Coop Agreement
Idaho State
University

THESIS/
REPORTS

AMMAN,
G.D.

The Effect of Philoem Thichness on In Laboratory-Reared Mountain Pine Beetles

FINAL REPORT FOR RESEARCH AGREEMENT
#INT-22-C-4-INT168
Idaho State University
FS Contact: Jessee Logan
Co-op Contact: M. W. Stock

THE EFFECT OF PHLOEM THICKNESS ON HETEROZYGOSITY IN LABORATORY-REARED MOUNTAIN PINE BEETLES

(Dendroctonus ponderosae Hopkins, COLEOPTERA:SCOLYTIDAE)

G. D. Amman

UDSA Forest Service

Intermountain Forest and Range Experiment Station

507 25th Street

Ogden, Utah 84401

M. W. Stock

Department of Forest Resources

University of Idaho

Moscow, Idaho 83843

Outbreaks of the mountain pine beetle (Dendroctonus ponderosae Hopkins) cause widespread loss of pines, especially lodgepole pine (Pinus contorta Douglas) in western Canada (Safranyik et al. 1974) and the western United States (Amman et al. 1977). During epidemics, a single national forest may lose in excess of a million trees in one year; for example, 3.6 million lodgepole pine trees were killed on the Targhee National Forest, Idaho, in 1976 (Klein et al. 1979). Individual lodgepole stands may have 70 to 94 percent of the trees larger than 12.7 cm in diameter killed during the course of an infestation (McGregor et al. 1987).

As a result of this, factors influencing population fluctuations in this insect have received a great deal of attention. Several factors are believed to have important roles in regulating populations. These have centered around host resistance (Raffa and Berryman 1982; Shrimpton 1978), food availability (Amman 1972; Cole et al. 1976) and weather (Bentz et al. 1991; Safranyik 1978).

Natural populations contain high levels of genetic variation, and studies of this variation have provided insight not attainable by other techniques into the responses of a population to environmental factors. It is well established that heterozygosity in a population confers greater fitness in a natural, varying environment. Heterozygous populations are often able to maintain larger population sizes or biomass than less heterozygous populations (e.g., Beardmore 1983). These advantageous effects can, in most cases, be attributed to heterozygosity per se, not to the effects of specific gene combinations (Mitton & Grant 1984).

The advantage of heterozygosity is frequently expressed as higher survival under severe or stressful environmental conditions (e.g., Bryant 1974, 1976; Milkman 1978; Samollow & Soule 1983; Smith et al. 1975; Parsons 1971, 1987). Mountain pine beetles emerging from thin-phloem lodgepole pine were more heterozygous than beetles collected from thick-phloem lodgepole pine (Stock and Amman 1985), and overwintered pine engraver beetles (Ips pini) were significantly more heterozygous than their nonoverwintered counterparts (Gast and Stock 1992. Heterozygosity changes observed over time in several mountain pine beetle populations in the field corresponded with density fluctuations and, presumably, the environmental stressors that influence population numbers (Stock et al. 1992).

Laboratory studies with Drosophila and other organisms have helped elucidate the effect of selective agents on gene polymorphism at individual gene loci (e.g., Birley and Beardmore 1977). It therefore seems likely that changes in heterozygosity could be measured, under controlled laboratory conditions, to evaluate the effects of specific selection pressures on a mountain pine beetle population, and to help determine the relative contribution of these factors to population fluctuations in the field. The work described here, focuses on the association between food availablility and heterozygosity. Beetles in areas with healthy, unstressed trees with a thick phloem layer have more food and more space to produce galleries and offspring. Beetles attacking trees with thin phloem have less food and produce fewer offspring. We would therefore expect beetles reared in thin phloem to be more heterozygous than beetles reared in thick phloem under similar conditions. In this study, we monitored levels of heterozygosity over six consecutive

generations of mountain pine beetle reared in thick and thin phloem in the laboratory.

METHODS

Mountain pine beetles were obtained from logs cut on April 17, 1984, from four lodgepole pine trees that had thick (>2.5mm) phloem. The trees were located along the highway between Greendale Junction (close to Flaming Gorge) and Vernal, in northeastern Utah. This infestation was in epidemic status and near its peak in 1984. Green logs from live trees, used for rearing beetles in the laboratory, were obtained from the same area, and were selected on the basis of phloem thickness. Phloem thickness averaged 3mm on thick-phloem logs and 1.9mm on thin-phloem logs.

At the Ogden laboratory, the infested logs from the field were placed in rearing cages at room temperature (20 to 27°C). The overwintered larvae completed development and adults started to emerge on May 3, 1984. These adults were collected for genetic analysis for production of the next generation.

Beetles were sexed using characteristics of the seventh abdominal tergum (Lyon 1958). Pairs were introduced into predrilled holes 3mm in diameter and 25mm deep made in the phloem at the xylem interface. These holes were spaced 5cm apart around the basal circumference of the green logs. After the female, followed by the male, was introduced into a hole, a 2.5cm square piece of wire screen was stapled over the hole to keep the beetles from leaving. These newly infested logs were placed in constant temperature cabinets at 25°C where beetles made egg galleries and oviposited, and the new generation developed. When adult beetles of the new generation started to emerge, the logs were placed in individual rearing cages at room temperature. Beetles were collected as they emerged and kept separate by log so that mates could be selected from different logs, thus preventing sib matings. This procedure was followed for the F1 and F2 generations. Starting with emergence of the F2 generation, we placed green logs in the bottom of the temperature cabinets and allowed beetles to infest and mate at random. Logs were introduced into the cabinet one at a time and left until the amount of boring frass suggested a fairly high attack density had been achieved. After a log became infested it was placed in a rearing cage at a constant 25°C until adult emergence. This procedure was followed for the F3 through F6 generations.

Heterozygosity levels were measured in the original population and each of the following generations. Live samples of approximately 100 beetles from each generation and phloem thickness were mailed in petri dishes containing damp sawdust to the University of Idaho laboratory for electrophoretic analysis. Methods for electrophoresis of mountain pine beetles have been described by Higby and Stock (1982) and Bentz and Stock (1986). Heterozygosity at individual loci and average heterozygosity over all polymorphic loci (Nei 1975) were calculated for comparison of groups.

RESULTS AND DISCUSSION

Initially, we assayed a number of loci described in earlier studies of the mountain pine beetle, but, after the second generation, we focused our attention on the six loci (Est2, Aat1, Pep-la, Pep-gl, and Pgi) that were polymorphic in the base population or the F1 generation (Table 1). Of these six loci, the esterase locus (Est2) was most polymorphic and the phosphoglucose isomerase locus (Pgi) least polymorphic. Average heterozygosity over the six loci varied from 20 to 36 percent, high values reflecting use of polymorphic loci only. (When monomorphic loci are included in the calculation, heterozygosity in these and related bark beetles averages about 17%, Bentz and Stock 1986, Cane et al. 1991.)

Figure 1 shows changes observed in average heterozygosity at these loci over six generations in the laboratory. Average heterozygosity decreased over the first two generations, with beetles from thin phloem becoming more homozygous than those from thick phloem. We suspected that our method of pairing beetles and introducing them into the logs was at least partly responsible for this decline in heterozygosity. For the F1 and F2 generations, 81 to 125 handpicked pairs of beetles were introduced into fresh logs. Males and females were selected from different source logs in an attempt to maximize recombination and minimize inbreeding. Nevertheless, the observed decrease in heterozygosity and the low number of galleries produced (only seven in thin phloem and 31 in thick phloem for the F2 generation) indicated that a change in rearing methods was needed.

After the second (F2) generation, when beetles were permitted to pair up naturally and larger numbers of galleries were subsequently produced, average heterozygosity began to increase rapidly in both groups and continued to increase until the end of the study. By the F4 generation, heterozygosity in both groups was higher than in the original population. During the last four generations, heterozygosity in beetles from thin phloem increased faster than heterozygosity in beetles from thick phloem. At the end of the study, the population from thin phloem was more heterozygous than the population from thick phloem.

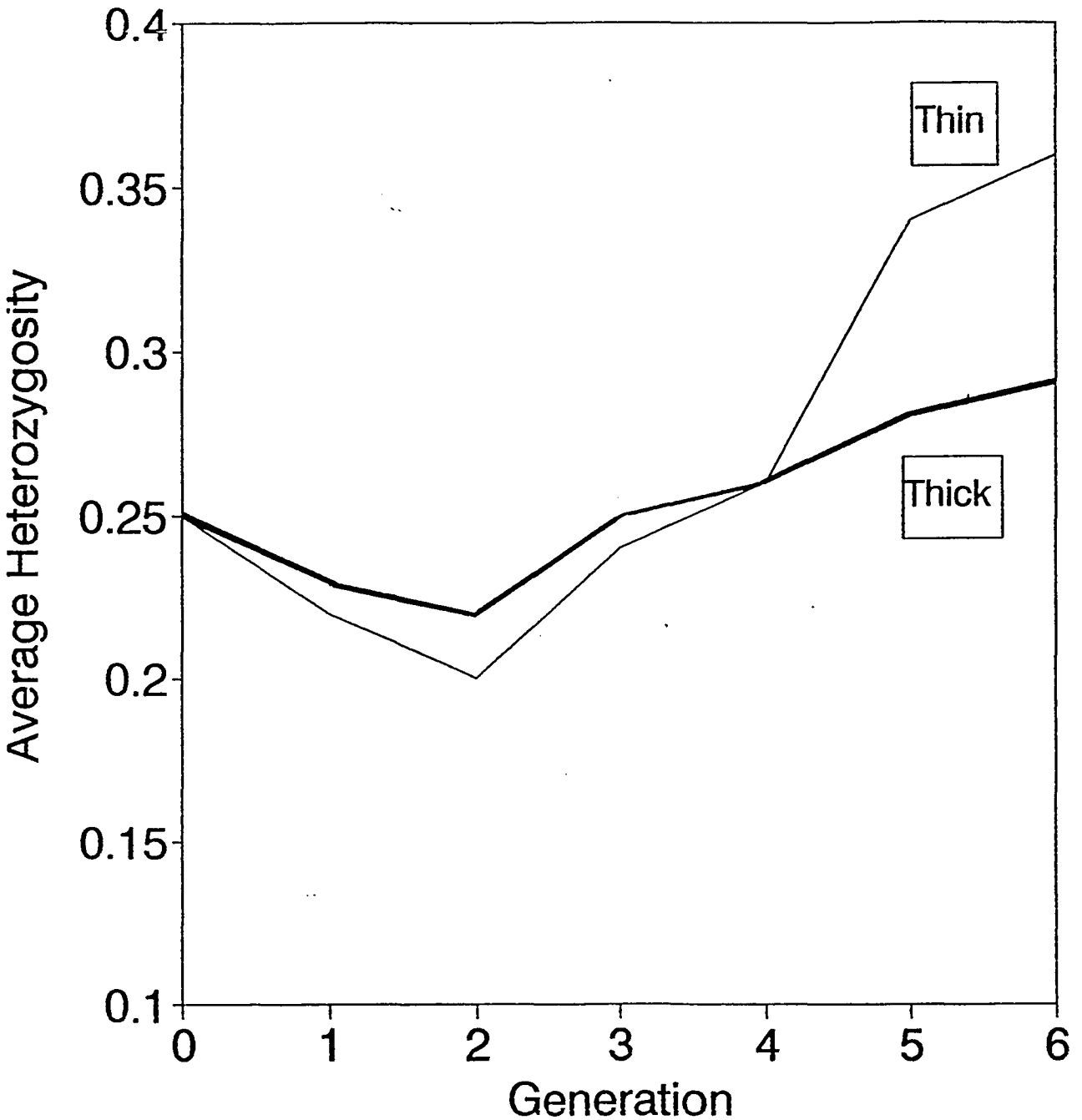
Overall, the results of this work supported our expectation that heterozygosity would increase faster over time in populations of beetles reared in the less hospitable environment of logs with thin phloem. It also suggests ways that mountain pine beetle rearing methods might be improved for experiments of this type. This work provides a basis for further laboratory experiments to evaluate the relative contribution of phloem thickness and other variables believed to affect survival and, consequently, population numbers in the mountain pine beetle.

ACKNOWLEDGMENTS

We thank Mike Perez for help rearing beetles and Sandra Gast Kegley for assistance with the laboratory work and computer analyses. Critical reviews of the manuscript were provided by ____. This study was supported in part by funds from The USDA, Forest Service, Intermountain Research Station, Odgen, UT 84401.

Table 1. Allele frequencies, heterozygosity (h), and average heterozygosity (H) for six consecutive generations of laboratory-reared mountain pine beetle.

Locus		Base Pop.	F1 Thn	F1 Thk	F2 Thn	F2 Thk	F3 Thn	F3 Thk	F4 Thn	F4 Thk	F5 Thn	F5 Thk	F6 Thn	F6 Thk
AAT	1	.77	.83	74	.82	.76	.64	.61	.65	.68	.58	.62	.61	.55
	2	.23	.17	26	.18	.24	.36	.39	.35	.32	.42	.38	.39	.45
	N	114	95	93	39	60	109	111	95	104	107	109	90	87
	h	.35	.28	.38	.29	.36	.46	.48	.45	.43	.49	.47	.48	.49
EST 2	1	.03	.03	.03	.02	.03	.05	.03	.04	.07	.05	.07	.07	.05
	2	.34	.36	.33	.38	.48	.29	.58	.41	.31	.37	.39	.31	.35
	3	.26	.26	.24	.38	.28	.27	.15	.27	.26	.32	.31	.24	.20
	4	.12	.13	.11	--	.04	.06	.12	.09	.08	.10	.06	.15	.14
	5	.23	.21	.27	.22	.16	.33	.10	.19	.28	.15	.16	.21	.24
	6	.01	.01	.01	.01	.01	--	.02	--	.01	.01	.01	.01	.02
	N	112	99	92	44	61	115	119	105	106	108	105	99	98
	h	.75	.74	.75	.66	.66	.73	.61	.71	.75	.73	.72	.77	.76
EST 4	1	.99	.99	1.0	.90	.97	1.0	.98	.99	.98	.99	.99	.98	1.0
	2	.01	.01	--	.10	.03	--	.02	.01	.02	.01	.01	.02	--
	N	118	100	100	44	61	120	120	110	110	120	120	100	100
	h	.02	.02	0	.18	.06	0	.04	.02	.04	.02	.02	.04	0
PEP gl	1	.91	.95	.94	.99	.94	.94	.89	.88	.91	.75	.99	.84	.91
	2	.09	.05	.06	.01	.06	.07	.12	.12	.09	.25	.10	.16	.09
	N	115	100	98	44	61	100	100	109	110	120	120	97	94
	h	.16	.09	.11	.02	.11	.11	.19	.21	.16	.37	.02	.27	.16
PEP 1a	1	.01	.02	.01	--	.01	.03	--	--	.01	--	.02	.25	.01
	2	.88	.91	.93	.99	.93	.94	.89	.91	.92	.67	.63	.38	.80
	3	.11	.07	.06	.01	.07	.04	.10	.09	.06	.33	.35	.38	.19
	N	115	90	90	44	60	116	113	106	109	86	89	97	98
	h	.20	.17	.13	.02	.13	.11	.19	.16	.15	.44	.48	.65	.32
PGI	1	1.0	.99	1.0	.99	1.0	1.0	1.0	1.0	.99	1.0	1.0	.99	1.0
	2	.01	.01	--	.01	--	--	--	--	.01	--	--	.01	--
	N	109	100	100	44	61	120	120	110	110	120	120	100	100
	h	0	.02	0	.02	0	0	0	0	.02	.02	0	0	0
H		.25	.22	.23	.20	.22	.24	.25	.26	.26	.34	.28	.36	.29


0.4
0.35
0.3
0.25
0.2
0.15
0.1
Average Heterozygosity
0
1
2
3
4
5
6
Generation
Thin
Thick

LITERATURE CITED

Amman, G. D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. J. Econ. Entomol. 65:138-140.

Amman, G. D., M. D. McGregor, D. B. Cahill and W. H. Klein. 1977. Guidelines for reducing losses of logdepole pine to the mountain pine beetle in unmanaged stands in the Rocky Mountains. USDA, For. Serv., Interm. For. and Range Expt. Sta. Gen. Tech. Rpt. INT-36, 19p.

Beardmore, J. A. 1983. Extinction, survival, and genetic variation. Pp. 125-151 in Schoenewald-Cox, C. M., S. M. Chambers, B. MacBryde, and W. L. Thomas (eds.). 1983. Genetics and conservation. The Benjamin/Cummings Publ. Co., Inc., Menlo Park, CA. 727p.

Bentz, B. J., J. A. Logan and G. D. Amman. 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera:Scolytidae) and simulation of its phenology. Canad. Entomol. 123:-1083-1094.

Bentz, B. J. & M. W. Stock. 1986. Phenetic and phylogenetic relationships among ten species of Dendroctonus bark beetles (Coleoptera:Scolytidae). Ann. Entomol. Soc. Amer., 79:527-534.

Birley, A. J. and J. A. Beardmore. 1977. Genetical composition, temperature, density and selection in an enzyme polymorphism. Heredity 39(1):133-144.

Bryant, E. H. 1974. On the adaptive significance of enzyme polymorphisms in relation to environmental variability. Am. Nat., 108:1-19.

Bryant, E. H. 1976. A comment on the role of environmental variation in maintaining polymorphisms in natural populations. Evolution, 30:188-189.

Cane, J. H., M. W. Stock, D. L. Wood & S. J. Gast. 1990. Phylogenetic relationships of Ips bark beetles (Coleoptera:Scolytidae): electrophoretic and morphometric analyses of Hopping's Group IX. Biochem. Syst. Ecol., 18(5):359-368.

Cole, W. E., G. D. Amman and C. E. Jensen. 1976. Mathematical models for the mountain pine beetle-lodgepole pine interaction. Envir. Entomol. 5:11-19.

Ferguson, A. 1980. Biochemical systematics and evolution. John Wiley and Sons, New York. 194p.

Higby, P. K. & M. W. Stock. 1982. Genetic relationships between two sibling species of bark beetle (Coleoptera: Scolytidae), Jeffrey pine beetle and mountain pine beetle, in northern California. Ann. Entomol. Soc. Amer., 75:668-674.

Klein, W. H., D. D. Bennett and R. W. Young. 1979. A pilot survey to measure annual mortality of lodgepole pine caused by the mountain pine beetle. USDA for. Serv. Intermtn. Region and Methods Applications Group, Rep. 78-4, 15p.

Lyon, R. L. 1958. A useful secondary sex characteristic in Dendroctonus bark beetles. Canad. Entomol. 90:582-584.

McGregor, M. D., G. D. Amman, R. F. Schmitz and R. D. Oakes. 1987. Partial cutting lodgepole pine stands to reduce losses to the mountain pine beetle. Canad. J. For. Res. 17:1234-1239.

Milkman, R. 1978. Selection differentials and selection coefficients. Genetics, 88:391-403.

Mitton, J. B. & M. C. Grant. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Ann. Rev. Ecol. Syst., 15:479-499.

Nei, M. 1975. Genetic variability in natural populations. Pp. 127-174 in Molecular population genetics and evolution. North Holland Research Monographs, Frontiers of Biology 40, American Elsevier Publ. Co., Inc., New York.

Parsons, P. A. 1971. Extreme-environment heterosis and genetic loads. Heredity, 26:479-483.

Parsons, P. A. 1987. Evolutionary rates under environmental stress. Evolutionary Biology, 21:311-347.

Raffa, K. F. and A. A. Berryman. (1982). Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. Environ. Entomol. 11:486-492.

Safranyik, L. 1978. Effects of climate and weather on mountain pine beetle populations. In: Berryman, A. A., G. D. Amman, and R. W. Stark (tech.eds.). Theory and practice of mountain pine beetle management in lodgepole pine forests. Sym Proc: April 25-27, 1978, Pullman, WA. Univ. of Idaho, Forest, Wildlife and Range Exp. Sta., Moscow, ID pp.77-84.

Safranyik, L.; Shrimpton, D. M.; Whitney, H. S. 1974. Management of lodgepole pine to reduce losses from the mountain pine beetle. Tech. Rep. 1. Victoria, BC: Canadian Forestry Service, Pacific Forest Research Centre, Department of the Environment: 24 p.

Samollow, P. B. & M. E. Soule. 1983. A case of stress related heterozygote superiority in nature. Evolution 37(3):646-649.

Shrimpton, D. M. 1978. Effects of lodgepole pine resistance on mountain pine beetle population. In: Berryman, A. A., G. D. Amman, and R. W. Stark (tech. eds.) Theory and practice of mountain pine beetle management in lodgepole pine forests. Sym. Proc.; April 25-27, 1978, Pullman, WA. Univ. of Idaho, Forest, and Wildlife and Range Exp. Sta., Moscow, ID. pp. 64-76.

Smith, M. H., C. T. Garten & P. E. Ramsay. 1975. Genetic heterozygosity and population dynamics in small mammals. Pp. 85-102. In: C. L. Markert (ed.), Isozymes, genetics, and evolution. Academic Press, New York.

Stock, M. W. & G. D. Amman. 1985. Host effects on the genetic structure of mountain pine beetle, Dendroctonus ponderosa, populations. Pp. 83-95 in: The

role of the host in the population dynamics of forest insects, L. Safranyik (ed.). Banff, Alberta, Canada. 1983.

Stock M. W., R. F. Schmitz, and G. D. Amman. 1997 (in press). Heterozygosity and density change in mountain pine beetle (Dendroctonus ponderosae) populations (Coleoptera: Scolytidae). In manuscript.